IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Sean A. McCarthy et al. Serial No.: Not Available Filed: Herewith For: Secreted Proteins and Nucleic Acids Encoding Them (Formerly "Method for Identifying Genes Encoding Signal Sequences") Attorney Docket No.: MBIO1997-018DV1ACN1(M)	Group Art Unit: Not Available Examiner: Not Available

Assistant Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT

Prior to examination, please amend the above-identified application as follows:

In the Title:

Please replace the title with:

"Secreted Proteins and Nucleic Acids Encoding Them"

In the Sequence Listing:

Please replace the title with:

"Secreted Proteins and Nucleic Acids Encoding Them"

Please remove from the applicant category (section <110>, as defined by 37 C.F.R. § 1.823(b)) the names "Kuranda, Michael Joseph", and "Bulawa, Christine Ellen".

In the Specification:

Please amend the specification as follows. For the Examiner's convenience, a marked up version of paragraphs in the specification is enclosed (Exhibit A), in which text added to these

claims is underlined and text deleted is struck through. A version of amended paragraphs in the specification as of the instant amendment is enclosed as well (Exhibit B).

On page 1, after the title, please insert:

Related Application Information

This application is a continuation of U.S. application serial number 09/436,183, filed November 8, 1999, which is a divisional from U.S. patent number 6,046,000, issued April 4, 2000, both of which are hereby incorporated by reference.

On page 10, please amend the four paragraphs beginning on line 11 to read as follows:

Figure 2 illustrates the nucleic acid sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of emxosb4a11.

Figure 3 illustrates the nucleic acid sequence (SEQ ID NO:3) and deduced amino acid sequence (SEQ ID NO:4) of emxosb4f08.

Figure 4 illustrates the deduced amino acid sequence of emxosb4a11 (SEQ ID NO:2) and its alignment (SEQ ID NO:14) to a portion of murine semaphorin F (SEQ ID NO:5).

Figure 5 illustrates the deduced amino acid sequence of emxosb4f08 (SEQ ID NO:4) and its alignment (SEQ ID NO:15) to a portion of a putative calcium binding protein (SEQ ID NO:6).

On page 12, please amend the paragraph beginning on line 27 to read as follows:

A yeast expression vector appropriate for use in the invention can be constructed as described below (Example 1, step 2) or from other suitable vectors. Examples of such vectors are described in, for example, Pouwels et al. (Cloning Vectors, Elsevier, New York, 1987 and Supplements); Rose et al., 1990, Methods in Yeast Genetics: A Laboratory Course Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; and Guthrie and Fink, eds., 1991, Guide to Yeast Genetics and Molecular Biology, Meth. Enzymol. 194, Academic Press, Inc. Harcourt, Brace Jovanovich, New York. An appropriate yeast expression vector for use in the

invention includes a suitable yeast promoter and transcription terminator (e.g., those of alcohol dehydrogenase; *ADH1*), and a yeast origin of replication (e.g. the 2μ origin). For those embodiments including a selection step in *E. coli*; at least an *E. coli* origin of replication, and one or more *E. coli* selectable markers such as drug resistance genes (e.g., genes conferring ampicillin, chloramphenicol, or tetracycline resistance) are generally included in the vector.

On page 18, please amend the paragraph beginning on line 19 to read as follows:

The first step in developing the *KRE9*-based signal peptide trapping system was construction of an appropriate yeast strain. Standard media and techniques appropriate for Saccharomyces were used (Rose et al., 1990, Methods in Yeast Genetics: A Laboratory Course Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Guthrie and Fink, eds., 1991, Guide to Yeast Genetics and Molecular Biology, Meth. Enzymol. 194, Academic Press, Inc. Harcourt, Brace Jovanovich, New York). The parent strain used for the construction was the haploid SEY 6210/*kre9*::*HIS* (*mat a*, *leu2-3*, *ura3-52*, *his3-Δ200*, *lys2-801*, *trp-*Δ901, *suc2-*Δ9) containing wild type *KRE9* on a *PRS* 316/*URA3* vector (Yscreen1; Brown and Bussey, 1993). This strain is maintained on SD/-his,-ura (1.7 g yeast nitrogen base without amino acids and ammonium sulphate (DIFCO), 5 g ammonium sulfate, 0.66 g -His/-Ura dropout powder (Clontech; Palo Alto, CA), 20 g dextrose, and 20 g BACTO-AGAR® brand solidifying agent per liter).

On page 21, please amend the paragraph beginning on line 14 to read as follows:

To generate short cDNA fragments, some of which would be expected to represent the 5' ends of mRNAs that contain signal sequences, random priming was employed rather than the oligo d(T) priming method suggested by Stratagene. The single-stranded cDNA was made double-stranded, DNA linkers containing a free EcoR I overhang were ligated to both ends of the double-stranded cDNAs, and the linker-adapted double-stranded cDNAs were then digested with Xho I to generate a free Xho I overhang at the 3' ends of the cDNAs. All steps were performed using reagents from the Stratagene ZAP cDNA synthesis kit according to the manufacturer's instructions.

Linker-adapted double-stranded cDNAs were size selected by gel filtration through SEPHACRYL® brand S-500 cDNA Size Fractionation Columns (Gibco BRL; Bethesda, MD: Catalog #18092-015) according to the manufacturer's instructions.

On page 21, please amend the paragraph beginning on line 29 to read as follows:

Size selected, double-stranded cDNAs were ligated to pBOSS1 which had been digested with EcoR1 and Xho1 and purified by agarose gel electrophoresis. Following overnight incubation at 16°C, the ligation reactions were extracted with phenol/chloroform and precipitated with three volumes of absolute ethanol. Following centrifugation and extensive washing with 70% ethanol, the precipitate was resuspended in 5 µl water, and 1 µl of the suspension was used to transform electrocompetent DH10B *E. coli* (Gibco BRL) according to manufacturer's instructions using a Bio-Rad electroporation apparatus. The transformation was titered by plating dilutions of electroporated bacteria on LB plates containing 100 µg/ml ampicillin. Once titered, the entire library was transformed, plated onto LB-ampicillin plates, and grown overnight at 37°C. The following day, bacteria growing on the plates were scraped into LB, and plasmid DNA was prepared using Qiagen MEGA COLUMNS® brand spin columns, following manufacturer's instructions (Qiagen; Santa Clarita, CA). DNA was quantitated spectrophotometrically and analyzed by agarose gel electrophoresis.

On page 23, please amend the paragraph beginning on line 15 to read as follows:

Plates were incubated for 2-4 days at 30°C or until colonies were apparent. Colonies were scraped from plates resuspended in 5 ml of YPD, and pooled in a 50 ml conical tube. Next, the cells were pelleted, washed once with water, and resuspended in 1 ml yeast lysis buffer. An equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) and washed glass beads were added to tube containing yeast cells. The mixture was vortexed vigorously for two minutes, spun in an Eppendorf microcentrifuge for 5 minutes, and the supernatant was transferred to a clean tube. To 40 µl DH10B electrocompetent cells, 0.5 µl of supernatant (as prepared above) was added, and mixed on

ice. Cells were electroporated using a Bio-Rad GENE PULSER II® brand electroporation system. One pulse was delivered at 2.5 kv, 25 μs, 100Ω in a disposable electroporation cuvette with a 0.1 cm gap (Bio-Rad; #165-2089). Following electroporation, 1 ml SOC was added, and the mixture was incubated with shaking at 30°C for 1 hour. Bacteria were plated on LB-ampicillin plates and incubated overnight at 37°C. The next day, individual colonies were inoculated into 1 ml of LB-ampicillin culture medium in 96-well plates and grown overnight with shaking. One hundred microliter samples were transferred to a new 96-well plate containing 100 μl 50% glycerol per well, and stored at -80°C. A portion of glycerol stock was used to inoculate fresh LB-ampicillin cultures. Following overnight growth, an AGTC (Advanced Genetic Technology Corporation; Gaithersberg, MD) plasmid preparation was performed and the plasmids isolated from each culture were sequenced from both ends to determine the presence and nature of inserts. The forward sequencing primer was 5'-GAGCAACGGTATACGGCCTTCCTT-3' (SEQ ID NO:12), and the reverse sequencing primer was 5'-GGGATATGCCCCATTATCCATC-3' (SEQ ID NO:13).

In the Abstract:

Please replace the title with:

"Secreted Proteins and Nucleic Acids Encoding Them"

In the Claims:

Please cancel claims 1-17 without prejudice and add claims 18-25 as follows:

--18. A polypeptide encoded by an isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or 3.

19. A polypeptide encoded by an isolated nucleic comprising an open reading frame of the nucleotide sequence of SEQ ID NO:1 or 3.

20. A polypeptide comprising the amino acid sequence of SEQ ID NO:2 or 4.

21. A polypeptide consisting of the amino acid sequence of SEQ ID NO:2 or 4.

22. The polypeptide as in any one of claims 18 to 21 further comprising a polypeptide which is heterologous to a polypeptide as in any one of claims 18 to 21.

23. An isolated polypeptide encoded by a nucleic acid molecule which hybridizes to the complement of the nucleic acid molecule consisting of the nucleotide sequence of SEQ ID NO:1 or 3 under conditions comprising washing in 0.2X SSC, 0.1% SDS at 42°C.

24. The isolated polypeptide of claim 23, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to the complement of the nucleic acid molecule consisting of the nucleotide sequence of SEQ ID NO:1 or 3 under conditions comprising washing in 0.1X SSC at 68°C.

25. An isolated polypeptide encoded by a nucleic acid molecule which hybridizes to the complement of the nucleic acid molecule consisting of the nucleotide sequence of SEQ ID NO:1 or 3 under conditions comprising hybridizing 50°C in Church buffer (7% SDS, 0.5% $NaHPO_4$, 1 M EDTA, 1% BSA), and washing at 50°C in 2X SSC.--

REMARKS

Claims 1-17 have been cancelled without prejudice. Applicant reserves the right to further prosecute the same or similar claims in the instant or in a subsequent patent. Claims 18-25 have been added. No new matter has been added, and Applicant submits that all of the claims are now in condition for allowance, which action is requested.

Applicants have changed the title of the present application to reflect the claimed subject matter, and have added related application information, to list applications to which the present application claims priority, and which are incorporated by reference. Applicants have amended the specification to remove reference to any Internet URLs. Applicants have also indicated trademark status by application of the "®" symbol, by capitalizing any use of a trademark, and by

accompanying a trademark with the generic terminology. Applicants have also changed *the names only* of the deduced amino acid sequences listed on page 10, beginning on line 11, for consistency's sake, to reflect the sequence names as they correctly appear elsewhere in the specification (e.g., on page 25, lines 14, 21, 26, and page 26, lines 2 and 8).

"Express Mail" mailing label number EL 791759248 US

Date of Deposit November 6, 2001

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231

Signature

Mary MacKinnon

Please Print Name of Person Signing

Respectfully Submitted,
MILLENNIUM PHARMACEUTICALS, INC.

By

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EXHIBIT A

MARKED UP VERSION OF PARAGRAPHS IN THE SPECIFICATION AS OF THE INSTANT AMENDMENT FILED NOVEMBER 6, 2001

On page 1, please amend the title to read as follows: ~~Method For Identifying Genes Encoding Signal Sequences~~ Secreted Proteins and Nucleic Acids Encoding Them

On page 1, after the title, please insert:

Related Application Information

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On page 10, please amend the four paragraphs beginning on line 11 to read as follows

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Figure 3 illustrates the nucleic acid sequence (SEQ ID NO:3) and deduced amino acid sequence (SEQ ID NO:4) of ~~exmosb4f08~~ emxosb4f08.

Figure 4 illustrates the deduced amino acid sequence of ~~exmosb4all~~ emxosb4a11 (SEQ ID NO:2) and its alignment (SEQ ID NO:14) to a portion of murine semaphorin F (SEQ ID NO:5).

Figure 5 illustrates the deduced amino acid sequence of ~~exmosb4f08~~ emxosb4f08 (SEQ ID NO:4) and its alignment (SEQ ID NO:15) to a portion of a putative calcium binding protein (SEQ ID NO:6).

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EXHIBIT B

AMENDED PARAGRAPHS IN THE SPECIFICATION AS OF THE INSTANT AMENDMENT FILED NOVEMBER 6, 2001

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On page 1, after the title, please insert:

Related Application Information

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